ORIGINAL ARTICLE

Sleep Consolidates Motor Learning of Complex Movement Sequences in Mice

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Introduction: Sleep-dependent consolidation of motor learning has been extensively studied in humans, but it remains unclear why some, but not all, learned skills benefit from sleep.

Aims and Methods: Here, we compared 2 different motor tasks, both requiring the mice to run on an accelerating device. In the rotarod task, mice learn to maintain balance while running on a small rod, while in the complex wheel task, mice run on an accelerating wheel with an irregular rung pattern.

Results: In the rotarod task, performance improved to the same extent after sleep or after sleep deprivation (SD). Overall, using 7 different experimental protocols (41 sleep deprived mice, 26 sleeping controls), we found large interindividual differences in the learning and consolidation of the rotarod task, but sleep before/after training did not account for this variability. By contrast, using the complex wheel, we found that sleep after training, relative to SD, led to better performance from the beginning of the retest session, and longer sleep was correlated with greater subsequent performance. As in humans, the effects of sleep showed large interindividual variability and varied between fast and slow learners, with sleep favoring the preservation of learned skills in fast learners and leading to a net offline gain in the performance in slow learners. Using Fos expression as a proxy for neuronal activation, we also found that complex wheel training engaged motor cortex and hippocampus more than the rotarod training.

Conclusions: Sleep specifically consolidates a motor skill that requires complex movement sequences and strongly engages both motor cortex and hippocampus.

Keywords: sleep-dependent consolidation, motor learning, sleep deprivation, rotarod, complex wheel.

Statement of Significance

Sleep benefits some types of memory and not others, but the reasons remain unclear. We employed 2 different motor tasks, the rotarod task and a novel complex wheel task, and found that sleep specifically consolidated motor learning exclusively in the latter. In both tasks, mice run on an accelerating device but only the wheel task requires acquisition of complex movements with high spatial accuracy. Immunocytochemical analysis of Fos expression revealed that compared to the rotarod task, the complex wheel task induces higher neuronal activity in motor cortex and hippocampus but comparable activity in other areas including medial prefrontal cortex and striatum. Thus, sleep specifically consolidates motor learning with complex movement sequences.

INTRODUCTION

The beneficial effects of sleep in motor learning[1–6] are well established in humans, and the evidence is compelling for motor sequence learning, in which subjects are asked to perform complex movement sequences as quickly and as accurately as possible. Specifically, numerous studies of sequence learning that used finger-tapping, finger-to-thumb opposition, and other paradigms[7] reported that nighttime sleep as well as a post-training daytime nap favored consolidation of motor skills and improved task performance in subsequent sessions.[1–6] Brain imaging studies have shed light on the interaction between hippocampus, striatum, and prefrontal cortex during learning and consolidation of procedural memory.[8,9] However, the mechanisms underlying the sleep-dependent refinement of motor skills are still poorly understood. Thus, the essential requisites that determine whether a learned skill will benefit from sleep remain unclear and controversial.[10–12] For instance, on the one hand, there is evidence that the explicitness of the sequence to be learned is critical for sleep-dependency.[10,11] On the other hand, several other studies found beneficial effects of sleep in motor adaptation tasks that require implicit learning.[13–15] There is also some evidence that more difficult tasks benefit more from sleep, but this conclusion was reached by comparing tasks that were all sleep-dependent.[16]

Sleep-dependent consolidation of motor skills is much less documented in animals. In the rotarod task, mice or rats learn to maintain their balance and run on a small rod that rotates at a constant acceleration, and the speed when the animal falls off the rod is recorded as the measure of performance.[17–23] Previous studies using one training session per day found that rotarod performance shows fast improvement within a session and a slower improvement across sessions. Intrasession improvement diminishes across days, and performance reaches a plateau within 3–5 days.[19,20,23] A recent study compared the next day improvement in rotarod performance in mice that were either sleep-deprived or allowed to sleep after training.[22] Both groups performed better the next day, but the improvement was reduced approximately by half (from 44% to 23%) in the sleep-deprived mice. However, that work could not establish whether sleep promoted fast, intrasession learning and/or offline consolidation. Very few other studies in rodents have used tasks that require the acquisition of complex movement sequences. One is the reaching task, in which rodents learn to approach a small opening in the front of the recording chamber, determine whether a sucrose pellet is available on the shelf, and, if so, reach through the opening to retrieve the pellet with the preferred paw.[24,25] In rats, 2 h of post-training sleep led to faster reaching movements relative to 2 h of sleep deprivation (SD), with no decrements in accuracy.[24] In mice instead, 5 h of post-training sleep did not provide an immediate advantage over an equivalent time of forced wake.[25] Mice that could sleep did show a delayed gain in performance 24 h after training, but improvement was measured across the entire session without teasing apart the offline consolidation from any additional learning during retest.[25] In summary, the evidence that sleep benefits motor skill learning and/or sequence learning is scant in rodents. Yet, the

characterization of sleep-dependent motor tasks in mice would pave way to the use of genetic, molecular, and electrophysiological approaches to understand how sleep benefits learning and memory.

Here, we aimed at clarifying whether sleep promotes specific forms of motor learning in mice and if so, whether it facilitates intrasession learning, offline consolidation, or both. We used 2 tasks, the rotarod task and a modified version of the "classical" complex wheel running task,[26–30] in which we trained mice to run on top of an accelerating wheel that lacks some rungs, rendering the rung pattern irregular and highly complex. Both tasks require the mice to run on an accelerating device and involve a short first training session (~1 h) without pretraining or food restriction. However, compared to the rotarod task, the complex wheel task has an additional motor sequence learning component, as the acquisition of the exact position of the paws, and the precise sequence of movements are required to run on the wheel. We find no evidence for sleep-dependent consolidation after rotarod training. By contrast, we show that the complex wheel task, which is more difficult than the rotarod task and leads to stronger activation of motor cortex and hippocampus, benefits from sleep. Thus, we provide, to the best of our knowledge, the first evidence of offline, sleep-dependent consolidation of sequence learning in mice and identify some of the factors that make a task sensitive to the effects of sleep.

METHODS

Animals

B6.Cg-Tg(Thy1-YFP)16Jrs/J mice (YFP-H, Jackson Laboratory) were maintained on a 12 h light/12 h dark cycle (lights on at 8:00 am) with food and water available ad libitum. YFP-H mice express yellow fluorescent protein (YFP) in a subset of cortical pyramidal neurons[31] and thus can be used to study the link between sleep and synaptic plasticity.[32–34] In total, we used 67 mice (52 males and 15 females) for behavioral experiments with the rotarod task, 188 mice (121 males and 67 females) for a complex wheel task, 4 mice (3 males, 1 female) for a regular wheel task and 15 additional male mice for Fos immunohistochemistry (4 sleeping controls, 3 mice for rotarod 20 trials, 4 for rotarod 40 trials, and 4 for complex wheel 20 trials) (Table S1). In each experiment, most, if not all, mice were litter-matched. All animal procedures and experimental protocols followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the licensing committee. Animal facilities were reviewed and approved by the institutional animal care and use committee (IACUC) of the University of Wisconsin–Madison, and were inspected and accredited by the association for assessment and accreditation of laboratory animal care (AAALAC).

Sleep Recordings and SD

Experiments were done in adolescent mice (P29-36, mostly P29-32) (Table S1). It was previously shown that 1-month-old YFP-H mice have consolidated sleep–wake patterns and homeostatic sleep regulation similar to adult mice.[33] Sleep and wake states were determined by continuous monitoring with infrared cameras (OptiView Technologies) starting at least 24 h before the first training session. This method cannot distinguish non-rapid eye movement (NREM) sleep from REM sleep, but it consistently estimates total sleep time with 90% accuracy.[32] Motor activity was quantified by custom-made video-based motion detection algorithms (Matlab), as previously described.[35] SD was enforced using 2 methods: (1) gentle handling, in which mice were touched with a cotton swab and (2) exposure to novel objects, in which toys and other objects of different shape, color, and texture were introduced in the cage. In both cases, mice were stimulated only when they appeared drowsy, assumed a typical sleeping position, and/or closed their eyes. Mice were never disturbed when they were spontaneously awake, feeding, or drinking. During SD (7 h), mice were awake 95.0 ± 0.36% of the time (SD with gentle handling, SDgh) and 93.7 ± 0.46% of the time (SD with novel objects, SDob). During the same 7 h, mice allowed to sleep were awake 28.4 ± 0.77% of the time.

Rotarod

Four individual accelerating rotarod systems (EZRod; Omnitech Electronics, Inc.) were used, and each system controlled separately. Prior to the first training, all mice were weighed. Mice were placed onto a stationary rod and acceleration began. The acceleration profiles were fast (0–100 rpm in 3 min) or slow (0–80 rpm in 5 min), with the fast protocol used in most experiments, as summarized in Table S2. The actual acceleration in SI units was 314 cm/min^2 and 150.7 cm/min^2 in fast and slow protocol, respectively. Time and speed when mice fell off the rod were automatically recorded. Sometime, a mouse unable to keep up with the increasing speed would grab the rod to stay on it without running. In these cases, we gently pushed the animal off the rod, and we counted these trials as well. Each training session included 20 or 40 consecutive trials. For every 10 trials, mice were returned to their home cage for a 5-min rest period, during which mice mainly groomed but never slept. Since backward running is more difficult than forward running, mice had to be forced to train in the second paradigm by using a homemade anti-flipping tool made of 2 parallel plastic boards with adjustable distance between them, which forced the mouse to maintain the backward direction (Figure 1, A). As in the previous study,[22] the acceleration profile of backward training was 0–50 rpm in 3 min.

Surgery

To mimic the experimental conditions of the previous rotarod study,[22] a subset of mice underwent surgery and was implanted with electroencephalography (EEG) electrodes. Mice were anesthetized with isoflurane (3–5% for induction, 1–2% for maintenance) and positioned in a Kopf stereotaxic apparatus. After the skull was exposed, two screw-type EEG electrodes were implanted over frontal cortex and cerebellum paying attention not to damage the pial membrane. EEG electrodes and skull were then wholly covered by dental cement. After the surgery, mice were returned to their home cage and left undisturbed for 24 h of recovery prior to the first rotarod session.

Complex Wheel Task

We modified the classical complex wheel task[26–30] by attaching a complex wheel to an individual accelerating rotarod system (Figure 3, A). To create a "complex" wheel, we used a running

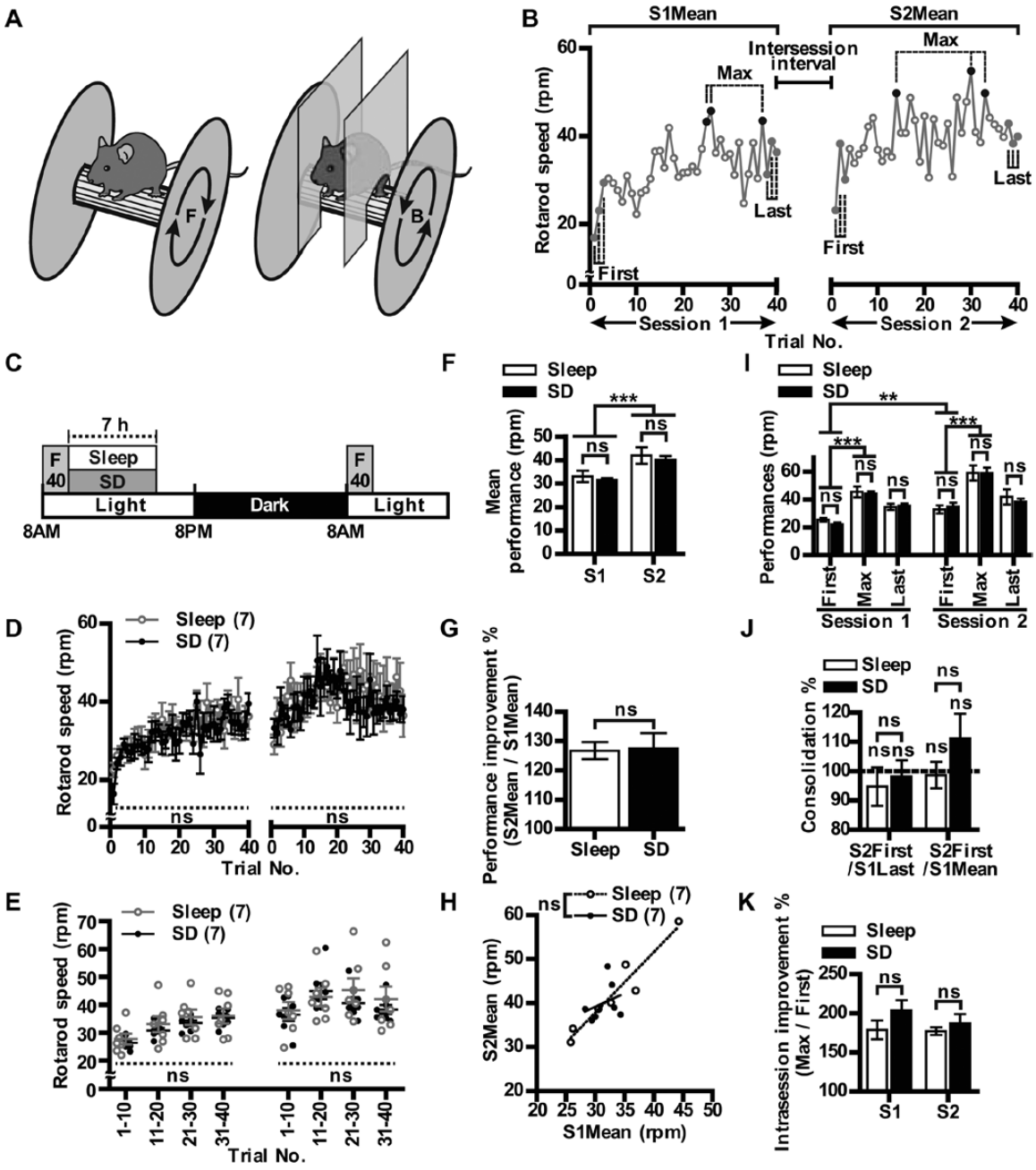

Figure 1—Rotarod task, measures of performance, and no evidence for sleep-dependent consolidation. (A) Schematic of the accelerating rotarod system with forward (F, left) and backward (B, right) running. In the backward running, the mouse is prevented from switching body position by an anti-flipping tool. (B) Intra- and intersession changes in performance in a single representative mouse, and the different parameters used to assess performance in each session: first 3, maximal 3, and last 3 trials, and mean of all trials. (C) Schematic of the experimental design. Mice were subjected to the first session of rotarod training at 8:00 am (S1, 40 trials) and then divided in 2 groups (n = 7 per group), depending on whether in the following 7 h they could sleep or were sleep deprived (SD) by gentle handling. The next day starting at 8:00 am mice were trained again (S2, 40 trials). (D) Performance values for each single trial after pooling all mice within each group. (E) Performance values for each single mouse after pooling values in groups of 10 trials. (F) Mean performance for each session. (G) Performance improvement across sessions. (H) Relationship between S1 Mean and S2 Mean for each mouse. Statistical significance was calculated by comparing the linear regression lines of Sleep and SD. (I) Performance measures for each session in the 2 groups. (J) Measure of offline consolidation. (K) Relative intrasession improvement. Values are expressed as mean ± SEM. $^{**}p < .01$, $^{***}p < .001$; 2-way repeated measures analysis of variance followed by Bonferroni post hoc test was used in (D–F, I, K), Student's t test in (G, J) and linear regression analysis followed by analysis of covariance in (H).

wheel that originally had 50 rungs, with rungs spaced 1.12 cm apart (wheel diameter 17.78 cm). These features are comparable to those of complex wheels previously used:[30] whose diameter, number of rungs, and space between rungs were 12.7, 38, and 1.05 cm, respectively. We removed 20 rungs to make 2 identical complex sequences of rungs in one rotation (Figure 3, A). Prior to the first training, all mice were weighed. At the beginning of the first session (20 trials), a mouse was placed onto the stationary complex wheel, and acceleration increased from 0 to 40 rpm over the course of 10 min (acceleration = 223.3 cm/min^2). To encourage the mouse to keep running on the top of the wheel, a fluffy sponge was placed in the back above the wheel with a small space (1–2 cm, depending on the body size of the mouse) between the wheel and the sponge (Figure 3, A and Supplementary Movie). Mice did not receive any habituation or pretraining using the complex or the regular wheel and thus usually spent some time exploring the device at the beginning of the first training session. If mice tried to escape from the chamber by grabbing the large disk connecting the rotarod to the motor system or by climbing up the sponge, they were gently placed back on top of the wheel. Mice sometimes also sniffed the sponge and squeezed their body below the sponge intentionally. In this case, the trial was stopped and repeated. These events were rare and occurred mostly at the lowest speed of the wheel (0–2 rpm). When the mouse could not keep up with the speed, the body was squeezed in the tiny space between the sponge and the wheel, and the trial was manually stopped by the experimenter by placing a hand in front of the infrared beam at the bottom of the chamber. In most cases, after each trial the mouse came back to the top of the wheel voluntarily, suggesting that the task was not stressful (Supplementary Movie). After the first 10 trials, mice were returned to their home cage for a 5-min rest period, during which they mainly groomed but never slept. Based on the median of the average performance in the first training session, mice were divided in fast and slow learners and the effects of sleep and SD were analyzed separately in each group, consistent with studies in humans.[36] To test the importance of complex sequences in learning, we also used a regular 50 rungs wheel as a control. Four mice received the regular wheel task according to the same protocol as the complex wheel task, with 2 sessions comprising 20 trials each, spaced 24 h apart. The acceleration profile was 0–40 rpm over the course of 10 min. A fluffy sponge was also placed in the back above the wheel, and each trial was manually stopped when the mouse was squeezed in the space between the sponge and the wheel.

Immunohistochemistry

The immediate early gene *c-fos* is a marker of neuronal activation, although the relationship between spontaneous neuronal activity and c-fos expression is not straightforward.[37] Many regions of the brain contain a large number of Fos-positive cells after animals have been awake for as few as 1–2 h, while after several hours of sleep Fos protein levels are undetectable in most, although not all, neurons.[38] To focus on task-specific neuronal activity, we aimed at reducing wake-related Fos expression by allowing mice to sleep for several hours. Specifically, mice were confirmed to have slept for more than 65% of the last 3 h and 85% of the last hour before the perfusion (sleep mice) or prior to the onset of training in the rotarod or complex wheel task (trained mice). Task training occurred between 5:30 pm and 7:15 pm, and each mouse was immediately killed after the task. Mice were deeply anesthetized with isoflurane (3–5%) and transcardially perfused with a flush of saline followed by 0.1 M phosphate buffer containing 4% paraformaldehyde. The brain was removed and postfixed in the same fixative overnight at 4°C. The brain was then cut into 40 µm sections using a vibratome, and tissue sections were subjected to immunohistochemistry or kept in 0.05 M phosphate-buffered saline (PBS) containing 0.05% sodium azide at 4°C until use. The sections were rinsed with PBS and then incubated in PBS containing 0.1% hydrogen peroxide for 30 min to inactivate endogenous peroxidases. After rinsing with PBS, the sections were incubated in blocking solution (PBS containing 3% normal goat serum and 0.1% triton X-100) for 1 hr and then overnight in blocking solution containing the primary antibody against c-fos (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were subsequently reacted with a biotinylated secondary antibody (BA-1000; Vector Laboratories, Burlingame, CA, USA) for 2 h and visualized using the avidin–biotin system (PK-4000; Vector Laboratories) and diaminobenzidine (SK-4100; Vector Laboratories). Sections were rinsed 3 times between each reaction, and all steps were done at room temperature. The sections were then dehydrated, coverslipped, and examined under a light microscope. To analyze Fos expression, each brain region of interest was first identified based on the Allen Mouse Reference Brain Atlas. Specifically, for each coronal section and area of interest (e.g., anterior cingulate, primary motor, and primary somatosensory), we measured on the Atlas mediolateral and dorsoventral extent, the latter subdividing the cortex in layers (layer 1, layers 2/3, layer 4 if applicable, and layers 5/6). We then created a region-of-interest mask based on these measures and applied it to each of our images to identify the borders of each cortical area. Cortical depth (from layer 1 to the white matter below layer 6) as measured using the Atlas matched well with that of our sections, so that we could designate each area consistently as shown in Figure 6, B. Within each designated cortical area, we then manually counted all Fos-positive cells. The caudate–putamen was subdivided in 2 parts (medial and lateral), and cell counting was done separately for each of them. In the hippocampus, Fos-positive cells were counted in CA1, CA3, and dentate gyrus, and their number was expressed per length (in millimeters) of each hippocampal region.

Statistics

Data are expressed as mean values ± SEM. All data sets were subjected to Shapiro–Wilk test to examine the normality of distribution prior to each statistical analysis. Statistics were calculated by using paired or unpaired 2-tailed Student's *t* test, 1-way analysis if variance (ANOVA) with a post hoc Tukey test, 2-way repeated-measures ANOVA with a post hoc Bonferroni test, linear regression test, analysis of covariance, Pearson test, or Spearman rank test, with IBM SPSS statistics 22. Student's *t* test and Pearson test were used for data sets with normal distribution, and Spearman rank test was used for data sets with non-normal distribution. ANOVA was used in most statistical

analyses based on its robustness against violation of normal distribution.[39]

RESULTS

Assessment of Rotarod Task and Definition of Measures of Performance

First, we used a training routine employed in previous studies.[22] Specifically, 1-month-old YFP-H mice (n = 7) were trained in forward rotarod running (Figure 1, A, left) in 2 morning sessions, S1 and S2, spaced 24 h apart. Between sessions, mice could sleep ad libitum. Each session included 40 trials, with the rod accelerating from 0 to 100 rpm over the course of 3 min.[22] Figure 1, B shows the changes in performance in one representative mouse across the first (S1) and the second (S2) session. Within each session, there was some variability from one trial to the next, and performance in the last trials tended to decrease and to be more variable, perhaps due to fatigue. Since mean performance measured by averaging all trials in a session does not fully capture variability and fatigue, we also measured performance across the first 3 trials (First), the best 3 trials (Max), and the last 3 trials (Last). Moreover, we used the ratio between average performance in S2 and S1 (S2 Mean/S1 Mean) to calculate the performance improvement across sessions, and the ratio Max/First in each session to assess intrasession improvement. Finally, to test for offline, across session consolidation, we used 2 measures, S2 First/S1 Last and S2 First/S1 Mean. The first measure represents the most direct comparison of performance before and after sleep, while the second measure controls for inter-trial variability and the potential issue of fatigue at the end of the session. Both measures were used to assess offline consolidation within and across groups.

No Effects of Sleep in the Consolidation of the Rotarod Task Using Various Experimental Conditions

In the first experiment, we compared the performance of mice that could sleep between the 2 sessions with that of mice that were sleep deprived by gentle handling for 7 h following S1 (7 mice/group; Figure 1, C). Similarly to a previous study,[22] mice of both groups improved in S2 relative to S1. However, contrary to the previous report, we found no difference between the 2 groups in any of the parameters that were assessed, including the overall profile of the learning curve (Figure 1, D and E): Mean, First, Max, and Last performance in each session (Figure 1, F–K). Most crucially, neither group showed evidence of offline consolidation (Figure 1, J).

In the second experiment (Figure S1, A) one sleep group (n = 7 mice) was compared to 2 SD groups, one kept awake by gentle handling (SDgh, n = 5) and the other by exposure to novel objects (SDob, n = 5), which in our experience is a more physiological and effective method of SD.[32,35] We reasoned that in the first experiment with 40 trials, mice may have learned the task well enough to mask a clear effect of sleep loss. Thus, in this experiment, each session was limited to 20 trials. Time of training and duration of SD instead were not changed (Figure S1, A). Again, all 3 groups improved their performance over the course of training, with no differences across groups in any of the examined parameters (Figure S1, B–F), although in the SDob group mean offline consolidation reached significance (Figure S1, F).

So far, all experiments used a fast acceleration profile, from 0 to 100 rpm in 3 min, which is the same used in a recent study[22] but faster than the one employed in other reports.[20,40] Thus, we also trained mice using a slower acceleration profile (from 0 to 80 rpm in 5 min). Moreover, mice were first trained at 8:00 am, as usual, but S2 occurred immediately after 7 h of either SD (SDgh, n = 3 or SDob, n = 4) or undisturbed sleep (n = 4) to evaluate more immediate effects of sleep loss on learning (Figure S1, G). Again, all mice improved their performance (Figure S1, H–L), and in fact, mean improvement across sessions was significantly greater after SDob than after sleep (Figure S1, J), and offline consolidation was larger in either SD group than in the sleep group (Figure S1, L), possibly because mice tested immediately after SD were more alert and vigilant due to the stimuli used to keep them awake. Notably, despite the slower acceleration profile, performance measures in all 3 groups were comparable to those in mice that received training with the higher acceleration profile.

In the previous study, mice underwent surgery for EEG recording and two photon imaging, and the first rotarod training was given 24 h later,[22] when recovery from anesthesia and surgery may have been incomplete. Since this condition of "stress" may have helped to unmask the negative effects of SD, 2 other groups of mice underwent surgery for implant of EEG electrodes and 24 h later received the first session of rotarod practice. Afterwards, they were again divided into a sleep group (n = 3) and an SD group (n = 3, Figure S1, M). Despite the surgery, we found no differences in performance between the 2 groups, or their measures of learning and consolidation were in the range of those of intact mice (Figure S1, N–R).

Mice are nocturnal and tend to be asleep mostly during the day and be awake spontaneously mostly during the night. Thus, in another experiment, we assessed the effects of spontaneous wake by scheduling the first training session at the end of the light phase, followed by S2 24 h later (Figure S2, A). As expected, in the dark period immediately following S1 mice spent the majority of the time awake (wake as percentage of total time, 64.0 ± 1.9 in the first 4 h, 60.2 ± 2.4 in the first 7 h after the end of training). Overall levels of performance in S1 and improvement in S2 did not differ from those seen in the sleeping mice used in the previous experiments (Figure S2, B–F). Thus, in our experimental setup, improvement in performance in the rotarod task occurred with a similar time course and to the same extent independent of whether after the first training mice were asleep, forced to stay awake, or spontaneously awake. Moreover, this improvement in performance was present in all groups when comparing mean speed across sections. By contrast, offline consolidation (S2 First/S1 Mean) was rarely seen: in fact, it was not observed in any of the sleep groups and was present only in one SD experiment, when mice were tested immediately after SD (Figure S1, L).

No Effects of Sleep in Learning the Rotarod Task or in the Consolidation of the Task in the Presence of Interference

To determine whether sleep loss may affect the ability to learn the rotarod task, rather than impair the consolidation process

following learning, we performed 7 h of SD prior to S1 (pSD, Figure S2, G). Overall performance in S1 was slightly better in the pSD mice (n = 4) relative to the sleeping controls (n = 7, Figure S2, H) although the difference did not reach statistical significance (Figure S2, I; Sleep* S1 = 33.09 ± 2.45 rpm, pSD S1 = 38.63 ± 3.01 rpm). By contrast, performance improvement across sessions was significantly lower in the pSD group, likely due to the high performance in S1 (Figure S2, J). Overall, all performance measures in S2 did not differ between the 2 groups (Figure S2, I, K, L).

Next, we tested whether the consolidation of forward training would be impaired when backward training occurred just a few hours after the first session of forward running, presumably interfering with its consolidation. Since human studies suggest that sleep may help consolidation especially in conditions of interference,[3] we reasoned that this protocol may help unmasking the negative effect of sleep loss that we were unable to detect so far. Thus, 2 groups of mice were used: the sleep group (n = 5) slept for ~4 h after forward learning, then received backward training and was allowed to sleep again ad libitum, while the sleep-deprived group (n = 6) was kept awake between forward and backward training and for 2 h after backward training (Figure S2, M). As in a previous study,[22] backward training was implemented by using an anti-flipping tool that forced mice to run in the "wrong" direction (Figure 1, A, right). We found no evidence that backward training interfered with the consolidation of forward running, even when it was associated with sleep loss. Again, all mice learned, and motor learning and performances in all measures did not differ between the 2 groups (Figure S2, N–R) and were comparable to those seen in our previous experiments with forward training alone. Therefore, we did not find any deteriorating effects of SD in the rotarod task even when SD preceded S1 or was coupled with interference.

To increase statistical power, we also plotted all the data from experiments that shared the same number of trials, 40 (Figure 2, A) or 20 (Figure 2, B), but still found no evidence for any change between the 2 groups in the time course of performance improvement, either within or across sections. We then tested the relationship between mean and late performance in S1 and mean and early performance in S2 using data from all the mice (Figure 2, C, E and G). Large interindividual variability was present, but there was also a highly significant correlation, in all the groups, between performance in S1 and S2. Thus independent of sleep, high performance during the first training was more likely associated with high performance in the following session (Figure 2, D). Note also that offline gains, measured by comparing the performance at the beginning of S2 (S2 First) with either the average or last performance of S1 (S1 Mean or S1 Last), were not present in the sleep group but occurred in SD mice (Figure 2, F and H). This gain, however, was driven by the SD mice of one single experiment (Figure S1, G–L).

To understand why we could not replicate the results of the previous study that found beneficial effects of sleep in rotarod performance, we estimated performance means during the first training session in the mice of that study (based on their Figures 3, C and S5)[22] and compared them with those of our

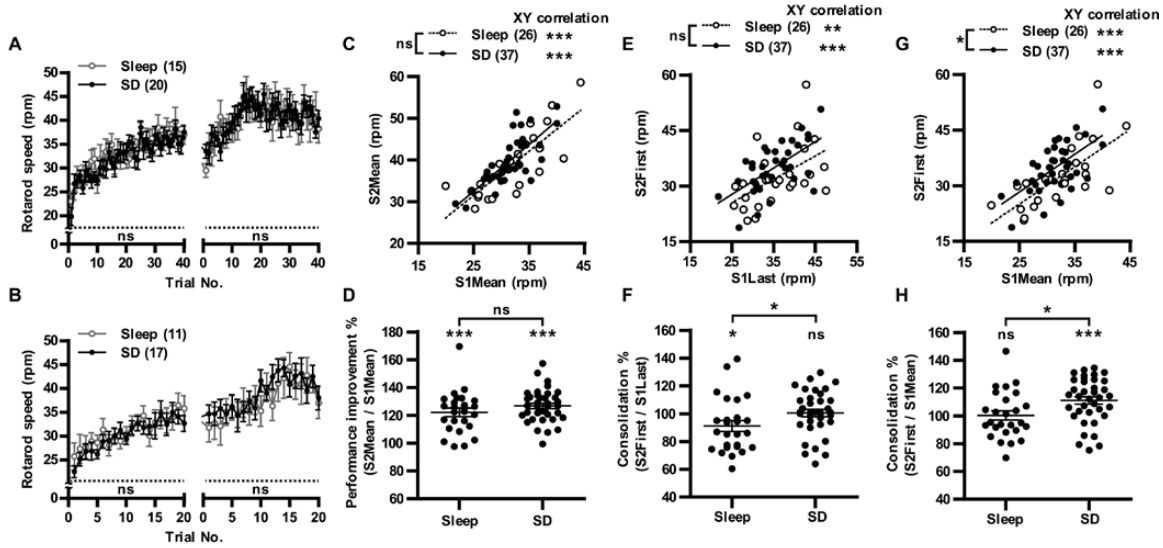

Figure 2—Overall analysis of rotarod learning. (A) Pooled data of all experiments with 40 trials (Figures 1, C, S1, M and S2, A, M). The experiment in which sleep deprivation was done prior to S1 is excluded. (B) Pooled data of all experiments with 20 trials (Figure S1, A, G). Statistical significance was calculated by comparing SD mice and sleeping controls in each session. (C, E, G) Relationship between S1 Mean and S2 Mean (C), S1 Last and S2 First (E), or S1 Mean and S2 First (G) for each mouse shown in A and B. Statistical significance was calculated by comparing the linear regression lines of sleep and SD. (D) Performance improvement across sessions for each mouse shown in A and B. Comparison between S2 Mean and S1 Mean within each group is indicated above each plot. (F, H) Consolidation of motor learning in each mouse assessed by using 2 measures, S2 First/S1 Last (F) and S2 First/S1 Mean (H). Comparison between S2 First and S1 Last or S1 Mean within each group is indicated above each plot. Values are expressed as mean ± SEM. *p < .05, ***p < .001; 2-way repeated measures analysis of variance followed by Student's t test was used in (A, B), linear regression analysis, analysis of covariance, and Spearman rank correlation test in (C, E, G), and Student's t test in (D, F, H).

mice. Mean performance in S1 was 32.2 rpm for their sleep mice (n = 5), which is very similar to that in our sleep mice (see Figure S3, A), while their SD mice (n = 7) had a mean performance in S1 of 22.4 rpm, a value that is lower than ours (Figure S3, A). Thus, SD mice in the previous study may have been on average poor performers, and performance in the 2 groups may not have been well balanced. Yet, in our own data, we found a strong correlation between mean performance in S1 and S2 (Figure 2, C) but not between mean performance in S1 and overall improvement across sessions (Figure S3, B). Thus, mice with low performance in S1 do not necessarily show low performance improvement across sessions. In summary, we do not have any obvious explanation for the discrepancy, but laboratory environment affects mouse behavior, and there may be subtle differences in the way the same task is implemented across laboratories.[41,42] Finally, rotarod performance in mice was previously shown to be negatively correlated with body weight,[43,44] while we found no correlation between body weight and motor performance (Figure S3, C). However, our mice were smaller (13–21 g), and our training protocol (40 trials) was more demanding than in previous studies, which used 1 single[43] or 3 trials per day.[44] Thus, intense learning may have masked any effect of weight. There is also conflicting evidence about sex differences in rotarod performance,[45,46] but in our experiments, males and females performed at similar levels (Figure S3, C).

Sleep Consolidated Motor Learning in the Complex Wheel Task

Next, we tested whether sleep facilitates the consolidation of complex motor skills that include sequence learning. With this aim, we developed a modified version of the complex wheel task by attaching a complex wheel to the device used to run the rotarod task (Figure 3, A and Supplementary Movie). As described in the Methods section, our version differs from the classical complex wheel task[26–30] in that mice are forced to run on top of the wheel rather than inside. To increase the chance to see sleep-dependent effects, mice were not pretrained, and intense training occurred within a limited time frame. Specifically, each training session contained 20 trials and the acceleration was 0–40 rpm over the course of 10 min. The measures of performance were the same used in the rotarod experiments to compare the results obtained with the 2 tasks (Figure 3, B).

In the first experiment, mice received the first training at 8:00 am and were then divided into a sleep group and an SD group that was kept awake by gentle handling for 7 h starting immediately after S1. All mice received S2 at 8:00 am the next day (Figure 3, C, morning-to-morning paradigm). Studies in humans found large inter-individual variability in learning motor tasks and differential effects of sleep in fast and slow learners.[36] From the very beginning of the study, we noticed that our mice also varied widely in their ability to perform the task. Thus, consistent with studies in humans, we used the median of the average performance in S1 to divide the mice in fast and slow learners, and studied the effects of sleep separately in the 2 groups (Figure 3, D). We first describe all the results for the fast learners and later (Figure 5) discuss the slow learners.

Among the fast learners in the morning-to-morning paradigm, sleep mice showed higher performance in S2 than SD mice, especially in the first half of the session (Figure 3, D and E). Specifically, sleep mice had higher mean performance (Figure 3, F), higher performance improvement across sessions (Figure 3, G and H), and higher first and max performance (Figure 3, I) than SD mice. Crucially, sleep mice, but not SD mice, were also significantly better at the beginning of the second session relative to their own mean performance in the first session (ratio S2 First/S1 Mean), resulting in a significant difference between the 2 groups (S2 First/S1 Mean, Figure 3, J). Results using the second measure of offline consolidation showed a similar trend, which, however, did not reach significance (S2 First/S1 Last; p = .116, Student's t test; Figure 3, J). Intrasession improvement instead was not significantly different between the 2 groups (Figure 3, K). Of note, performance improvements were not found when another group of mice (n = 4) run on a regular wheel without any pretraining: in this case, mice showed high performance (~10 rpm) from the very beginning of the first training session without any improvement across trials (Figure S4, A–C) or across sessions (Figure S4, D). Maximal performance in S1 (S1 Max) was not significantly different from initial performance (S1 First) (Figure S4, E), indicating lack of intrasession improvement.

Sleep-dependent Consolidation in the Complex Wheel Task Confirmed in Same Day Paradigms

To test whether sleep-dependent consolidation in the complex wheel task occurs within a few hours after the first training session, other groups of mice received S1 at 8:00 am and S2 immediately after 7 h of either sleep or SD by gentle handling (Figure S5, A, morning-to-afternoon paradigm). In this case, fast learners of both groups showed very similar performance in both sessions, in all measures (Figure S5, B–I). We noticed, however, that some sleep mice appeared drowsy at the beginning of S2, most likely because their sleep was abruptly terminated to start S2, suggesting that as in humans, sleep inertia may have masked the beneficial effects of sleep.[47–50] Consistent with this hypothesis, in the sleep group we found a positive correlation between time spent awake during the last hour before S2 and either performance improvement across sessions or S2 Mean performance (Figure S5, J, K). This positive correlation was not found using the previous morning-to-morning paradigm (Figure S6, A–C).

To avoid sleep inertia in the next experiment, sleep mice were allowed to sleep 9 h, instead of 7 h, and had 30 min of exposure to novel objects prior to S2 (Figure 4, A, morning-to-late afternoon paradigm). SD mice were kept awake by exposure to novel objects for the same amount of time (9.5 h). Using this study design, sleep mice did not appear drowsy at the onset of S2, and we found no correlation between time spent awake prior to S2 and performance in S2 (Figure S6, D–F). Consistent with the morning-to-morning experiment, among the fast learners sleep mice showed higher performance than SD mice in all S2 measures (Figure 4, B–G). Moreover, sleep mice showed significant offline consolidation, both relative to their own performance in S1 and as compared to SD mice, and did so using both measures of offline consolidation (Figure 4, G).

Next, to exclude the possibility that SD mice showed lower performance because of fatigue, we left all mice undisturbed

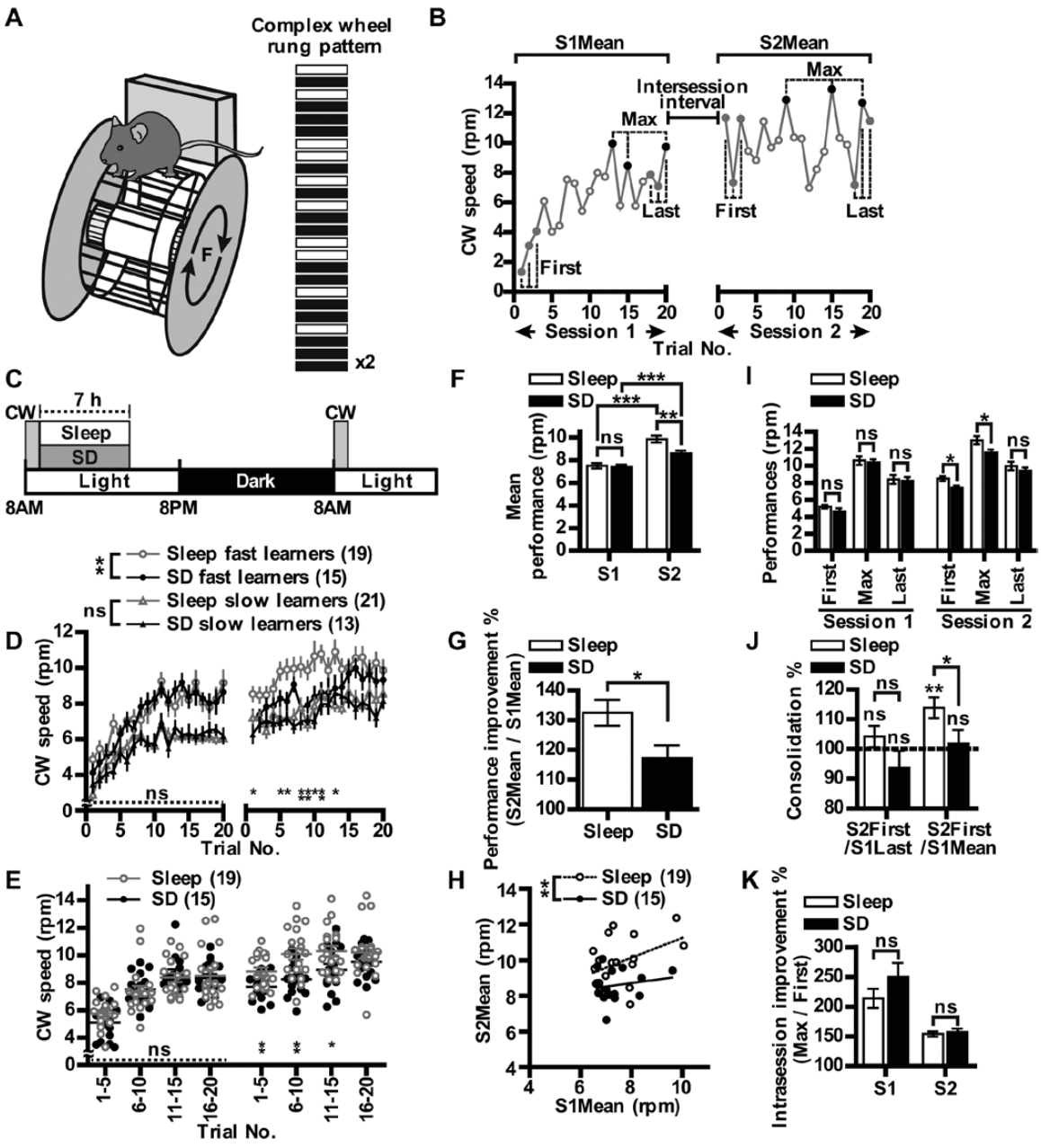

Figure 3—Sleep-dependent consolidation of motor learning using the complex wheel task: next day experiments. (A) Schematic and rung pattern of the complex wheel (CW). (B) Intra- and intersession changes in performance in a single representative mouse, and the different parameters used to assess performance in each session: first 3, maximal 3, and last 3 trials, and mean of all trials. (C) Experimental design. After the first session (S1, 20 trials) at 8:00 am, mice were divided in 2 groups depending on whether in the following 7 h they could sleep or were sleep deprived (SD) by gentle handling. The next day starting at 8:00 am mice were trained again (S2, 20 trials). (D) Performance of fast and slow learners in the sleep and SD groups shown for each single trial. (E) Performance in sleep and SD mice pooled across 5 trials; in this and the following panels, only data from fast learners are shown. (F) Mean performance for each session. (G) Mean performance improvement across sessions. (H) Relationship between S1 Mean and S2 Mean in each mouse. Statistical significance was calculated by comparing the linear regression lines of S and SD. (I) Performance measures for each session in the 2 groups. (J) Offline consolidation of motor skills using 2 measures. (K) Relative intrasession improvement. Values are mean ± SEM. *$p < .05$, **$p < .01$, ***$p < .001$; 2-way repeated measures analysis of variance followed by either Bonferroni post hoc test or Student's t test was used in (D–F, I, K), Student's t test in (G, J) and linear regression analysis followed by analysis of covariance in (H). ns, not significant.

for ~5 h after 7 h of sleep or SD by gentle handling, and performed S2 1 h after lights off (Figure 4, H, morning-to-night paradigm). Fast learners of both groups showed similar amount of spontaneous wakefulness just prior to S2 (Figure S6, G–I), ruling out the possibility that SD mice were sleepy even in the dark phase due to the sleep loss in the previous light phase.

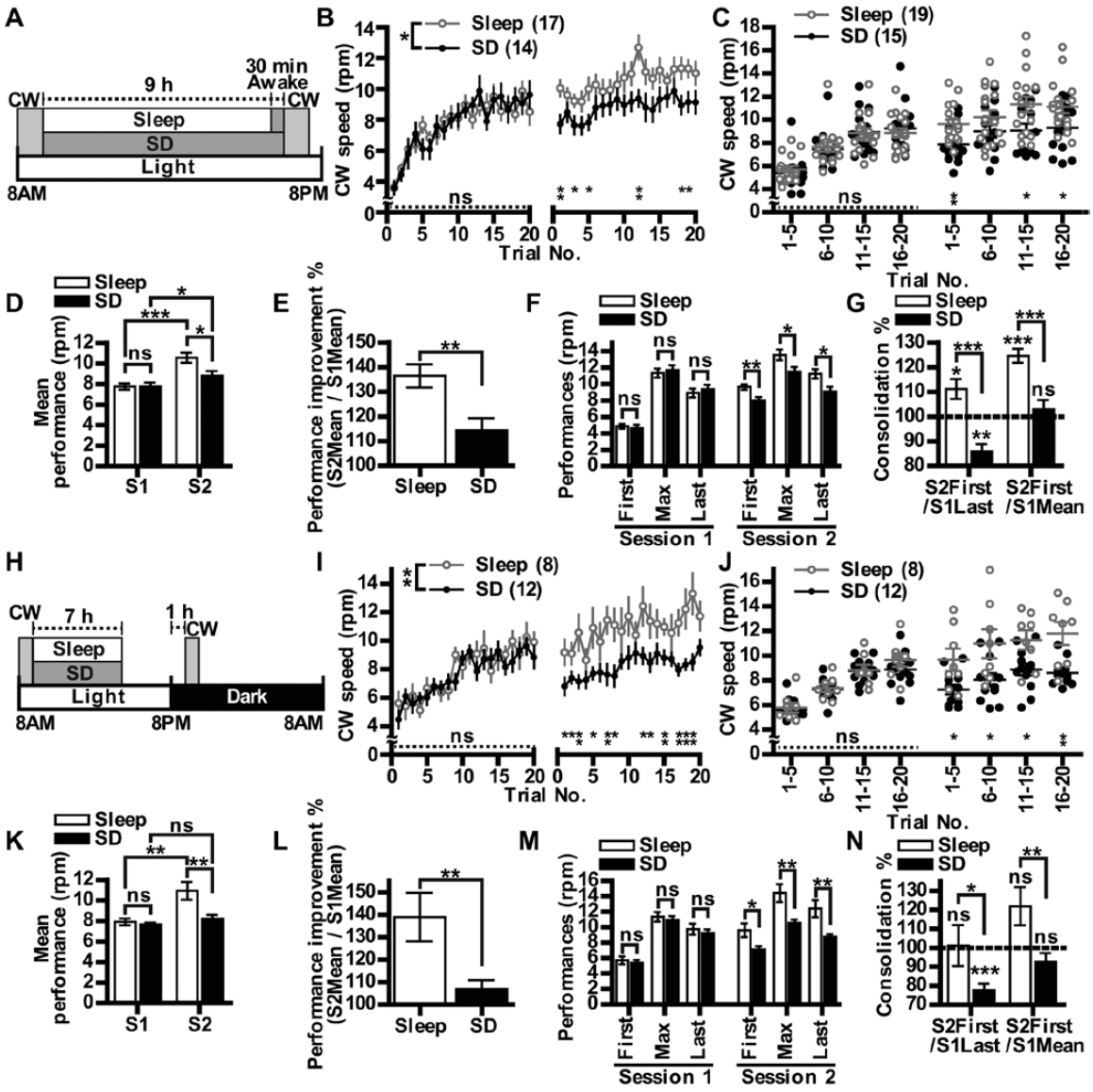

Figure 4—Sleep benefits motor learning in the complex wheel task: same day experiments. (A) Experimental design for the morning-to-late afternoon paradigm. After the first session (S1, 20 trials) at 8:00 am, mice were divided in 2 groups (Sleep $n = 24$; SD $n = 23$) depending on whether they could sleep or were sleep deprived afterwards. Sleep mice were left undisturbed for 9 h and received 30 min exposure to novel objects to dissipate sleep inertia, whereas SD group was deprived of sleep for 9.5 h by novel objects. The same day starting at 6:30 pm mice were trained again (S2, 20 trials). Only fast learners are shown (slow learners, $n = 7$ Sleep mice; $n = 9$ SD mice are shown in Figure 5). (B, C) Performance in the 2 groups shown for each single trial (B) and each 5 trials (C). (D) Mean performances for each session. (E) Performance improvement across sessions. (F) Performance measures for each session in the 2 groups. (G) Consolidation of motor skills using 2 measures. (H) Schematic of the experiment of the morning-to-night paradigm. Mice were subjected to the first session (S1, 20 trials) of complex wheel task at 8:00 am and then divided in 2 groups (18 Sleep and 18 SD) depending on whether in the following 7 h they could sleep or were sleep deprived by gentle handling. After 7 h, both groups were left undisturbed until they were trained again the same day at 9:00 pm (S2, 20 trials). Lights were always on in the training room. Only fast learners are shown (slow learners, $n = 10$ Sleep mice; $n = 6$ SD mice are shown in Figure 5). (I–N) Same measures as in B–G. Values are mean ± SEM. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$; 2-way repeated measures analysis of variance followed by either Bonferroni post hoc test or Student's t test was used in (B–D, F, I–K, M), and Student's t test in (E, G, L, N). CW, complex wheel; SD, sleep deprivation; S, session; ns, not significant.

Also with this paradigm, we found that sleep mice showed in S2 higher performance than SD mice in all measures (Figure 4, I–N). Moreover, sleep mice again showed significant offline consolidation as compared to SD mice using both measures (Figure 4, N).

Sleep Consolidates Motor Skill of the Complex Wheel Task Differently in Fast and Slow Learners

Next, we studied the effects of sleep on slow learners and compared them to those already described for the fast learners. To obtain a large and balanced number of animals in each

group (fast vs. slow, sleep vs. SD), we pooled the data from all the experiments except the morning-to-afternoon paradigm, whose results were confounded by sleep inertia. First, we tested whether at least some of the interindividual variability was due to differences in body weight and/or gender, and found that it was not (Figure S7).

Among the fast learners, there were 40 mice in the sleep group and 36 mice in the SD group (Figure 5, A). In both groups, performance in S1 predicted performance in S2 (linear regression analysis, sleep mice, $R^2 = 0.28$, $F(1,38) = 14.773$, $p < .001$; SD mice, $R^2 = 0.27$, $F(1,34) = 12.30$, $p < .01$). Moreover, both groups improved in S2 relative to S1, but sleep mice did so more than SD mice (Figure 5, B). Crucially, sleep mice showed offline consolidation when compared to SD mice. Specifically, at the onset of S2, sleep mice as a group maintained, but did not exceed, the peak performance reached at the end of S1, perhaps because they had already reached the highest scores afforded by a single training session (Figure 5, C and D). Performance in SD mice, however, was significantly worse at the onset of S2 than at the end of S1 (Figure 5, C and D), suggesting that sleep is required to prevent performance decay. Mean performance in S2 was positively correlated with time spent asleep during the 7 h after S1, while mean performance in S1 did not predict subsequent sleep quantity (Figure 5, E). Moreover, time spent asleep after initial training was positively correlated with one measure of offline consolidation (S2 First/S1 Mean), although not with the other (S2 First/S1 Last) (Figure 5, F), again perhaps due to a ceiling effect.

The slow learners included 42 sleep mice and 33 SD mice (Figure 5, G). Performance in S1 predicted performance in S2 only in sleep mice but not in SD mice (linear regression analysis, sleep mice, $R^2 = 0.25$, $F(1,40) = 7.062$, $p < .05$; SD mice, $R^2 = 0.05$, $F(1,31) = 1.583$, $p > .05$). Still, both groups improved in S2 relative to S1 (Figure 5, H). Slow learners also showed evidence of offline consolidation after sleep when compared to after SD, but for reasons different from those seen in the fast learners. Specifically, at the onset of S2 sleep mice as a group showed an offline gain, that is they exceeded the peak performance reached at the end of S1 (Figure 5, I and J). Unlike in the fast learners, however, SD did not lead to performance decay at the onset of S2 (Figure 5, I and J). In contrast to fast learners, time spent asleep after initial training did not correlate with measures of offline consolidation or mean performance in S2 (Figure 5, K and L).

Complex Wheel Training Activates More Neurons in Motor Cortex and Hippocampus than Rotarod Training

Both the complex wheel task and the rotarod task require the mice to run on an accelerating device, but in the former the mouse needs to learn complex movement sequences and relies more on the use of fine movements and visuospatial coordination. Thus, the 2 tasks are expected to rely on partially different patterns of neuronal activation. To identify them, we used Fos as marker of neuronal activity. To perform Fos immunohistochemistry, mice were perfused immediately following the first training session (Figure 6, A). Since wake is associated with widespread increased expression of Fos relative to sleep, all mice were allowed to sleep for several hours before the task to eliminate previous wake-related Fos expression.[37,38] Moreover, since mice take roughly half of the time to perform the same number of trials in the rotarod task relative to the complex wheel task, we compared animals that received 20 or 40 trials of rotarod training to those that received 20 trials of complex wheel training. Fos-positive cells were manually counted in the medial prefrontal cortex (prelimbic and anterior cingulate areas), primary and secondary motor cortices, primary somatosensory cortex, striatum, and hippocampus (Figure 6, B).

As expected, sleep controls showed negligible Fos expression in most of the brain regions (Figure 6, B–F). In all tested regions, mice that received 20 trials of rotarod training exhibited less Fos-positive cells than the other trained mice (Figure 6, B–F), probably because of the shorter awake time (Figure 6, G). Thus, we focused on the comparison between mice that underwent 40 trials of rotarod training and mice that received 20 trials of complex wheel training (all fast learners), as total awake time was similar in these 2 groups (Figure 6, G). Compared to rotarod training, complex wheel learning led to a significantly higher number of Fos-positive cells in supragranular and infragranular layers of primary motor area (Figure 6, E) and of secondary motor area (Figure 6, C and D), as well as in the CA1 region of the hippocampus (Figure 6, B and F). By contrast, no significant differences between the 2 groups were found in prelimbic and anterior cingulate cortex, dorsomedial and dorsolateral striatum, primary somatosensory cortex, CA3, and dentate gyrus of the hippocampus (Figure 6, C–F).

DISCUSSION

Sleep-dependent consolidation of motor skills is well documented in humans but much less so in animals. One of the few studies in mice recently suggested that sleep loss affects the consolidation of rotarod learning.[22] One of our goals was to build on these results and refine the evidence for offline consolidation. To follow the previous study as closely as possible, we used mice of the same transgenic line and age, as well as the same rotarod system and experimental design as reported previously.[22] However, to our surprise, mice improved equally well after sleep and after SD, independent of the method of SD (gentle handling vs. novel objects), time of testing (second training immediately after SD vs. the next day), length of training (20 vs. 40 trials), and whether they had undergone surgery 24 h before training. We also found that mice that were trained at the end of the light phase and then remained spontaneously awake for several hours improved as much as mice trained during the day and allowed to sleep after practice. For the first time, we also tested the effects of SD performed before the first training session as well as the effects of SD in mice trained in a more complex paradigm that involved forward running followed by backward running. In both experiments, sleep-deprived mice and sleeping controls performed equally well. Overall, there was no difference in mean performance between SD mice and sleeping controls in any of the 7 experimental designs we employed. For the first time, we also directly tested whether there was an offline gain in performance—sleep-dependent consolidation—by comparing performance at the beginning of the second session (S2 First) with either the last or the mean performance of the first session (S1 Last or S1 Mean). We found no evidence for

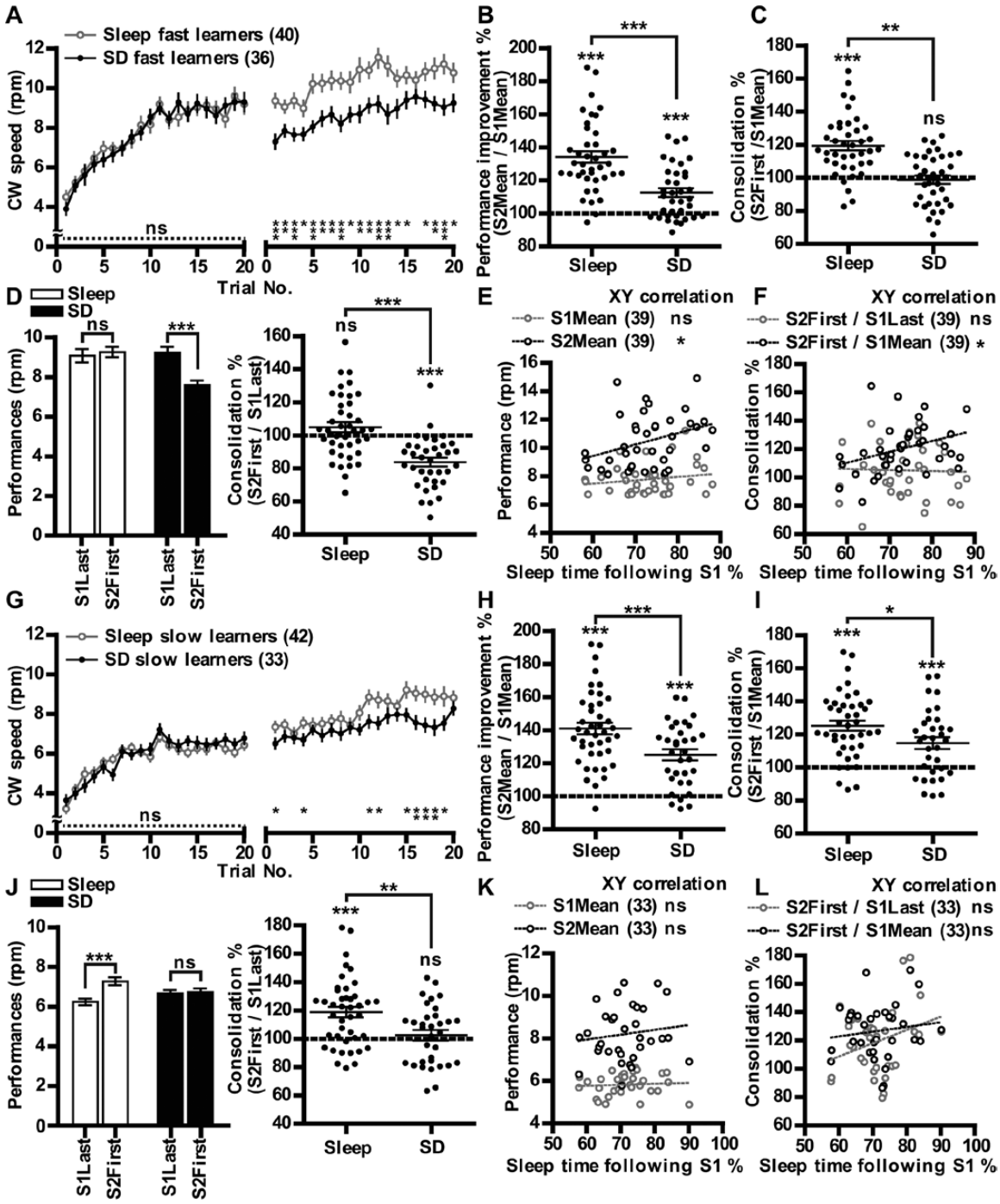

Figure 5—Comparison between fast and slow learners. Data were pooled across 3 experimental paradigms (morning-to-morning, to-late afternoon, and to-night) of fast and slow learners. The threshold to define fast and slow learners is based on the median of mean S1 performance across all pooled mice. (A–F) Fast learners. (A) Performance of each single trial. (B) Performance improvement across sessions. (C) Offline consolidation using the S2 First/S1 Mean ratio. (D) Offline consolidation using the S2 First/S1 Last ratio, with absolute performance values shown on the left panel. (E) Relationship between sleep time during the 7 h following S1 and mean performance of each session. Activity data of 1 mouse were missing. (F) Relationship between sleep time following S1 and offline consolidation using 2 measures (S2 First/S1 Last and S2 First/S1 Mean). (G–L) Same measures as in A–F for slow learners. Activity data of 9 mice were missing in (K, L). Values are mean ± SEM. *$p < .05$, **$p < .01$, ***$p < .001$; Comparison within each group is indicated above each plot in (B–D, H–J); 2-way repeated measures ANOVA followed by Student's *t* test was used in (A, G), Student's *t* test in (B–D, H–J), and correlation analysis was calculated in (E, F, K, L) either by Pearson or Spearman test based on normality of samples. CW, complex wheel; SD, sleep deprivation; S, session; ns, not significant.

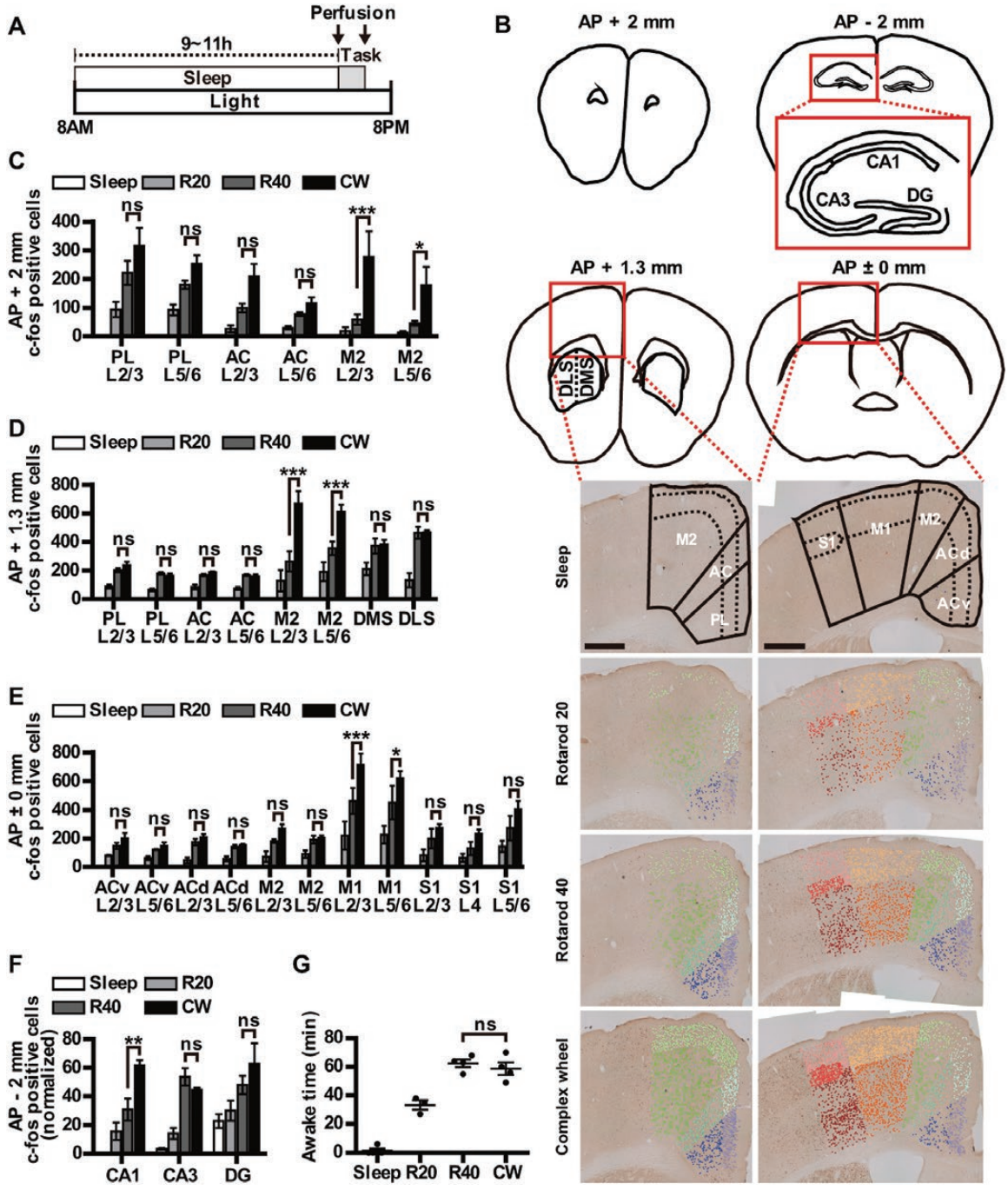

Figure 6—Complex wheel training leads to differential Fos expression in select areas relative to rotarod training. (A) Experimental design. Mice were confirmed to have slept before they were subjected to either immediate perfusion (sleep control, *n* = 4) or motor task training (rotarod 20 trials, R20, *n* = 3; rotarod 40 trials, R40, *n* = 4; complex wheel 20 trials, CW, *n* = 4, all fast learners). (B) Schematics of each brain area analyzed and representative results of Fos immunohistochemistry. The designated cortical area was determined based on the Allen mouse brain atlas. Each dot represents a Fos-positive cell identified by manual counting. Scale bars = 500 μm. (C–F) Number of Fos-positive cells in different brain areas corresponding to bregma +2 mm (C), +1.3 mm (D), ±0 mm (E), and −2 mm (F) AP. (G) Awake time is the time from when mice were taken out of their home cage until they were perfused. After being removed from their home cage, mice were either immediately perfused (sleeping controls), or trained for ~1 h and then perfused immediately after training. In the 3 groups of trained mice, awake time is mostly the time spent on the task. Values are mean ± SEM. **p* < .05, ***p* < .01, ****p* < .001; 2-way analysis of variance (ANOVA) followed by Bonferroni post hoc test was used in (C–F) and 1-way ANOVA followed by Tukey post hoc test was used in (G). PL, prelimbic area; ACv, anterior cingulate area ventral part; ACd, anterior cingulate area dorsal part; M1, primary motor area, M2, secondary motor area; DMS, dorsomedial striatum; DLS, dorsolateral striatum; S1, primary somatosensory area; DG, dentate gyrus; CW, complex wheel; ns, not significant.

better consolidation in mice allowed to sleep ad libitum either for 7 h or until the next day. If anything, we found some evidence for offline consolidation in a subset of SD mice, but this effect was limited to a single experiment. Finally, we found large interindividual variability in the way sleep and sleep loss affected this task. Thus, we conclude that sleep does not benefit motor learning in the rotarod task (Table S2), contrary to a previous report that was based on a small number of animals.

The complex wheel task demands close attention to the sequence of uneven rungs and requires complex movements of limbs and paws with high spatial accuracy. Therefore, it is perhaps not surprising that we found higher Fos expression, and thus presumably stronger neuronal activation, in a few select areas after complex wheel training compared to rotarod training. These areas included the supragranular and infragranular layers of primary motor cortex, the same layers that undergo plastic changes in response to training in the reaching task, including long-term potentiation-like strengthening of cortical connections and spine formation.[51,52] Higher Fos expression was also present in all layers of secondary motor cortex. This area in rodents is akin to the supplementary motor area of primates,[53,54] which has an established role in planning, initiation, and control of complex movements and motor routines.[55,56] Consistent with our data, another study in humans showed that regional cerebral blood flow in the supplementary motor area increased more during complex motor tasks than simple ones,[56] suggesting that the activity in this region reflects the complexity of the task. In our study, Fos expression was more pronounced in the rostral, compared to the caudal, part of secondary motor cortex (Figure 6, C–E), pointing to the former as the most critical area for learning or executing the complex wheel task. Moreover, a recent study in humans found that training in a finger tapping task led to an increase in sleep slow waves and fast spindles in the contralateral supplementary motor area, and these local sleep changes correlated with performance improvement.[57] Finally, Fos expression was also higher in the CA1 region of the hippocampus after complex wheel training relative to rotarod training (Figure 6, C–F). The hippocampus likely plays an important role in the initial phase of motor sequence learning, possibly because of its role in the promotion of higher order associations and processing of spatial information.[8] Moreover, some studies in humans have specifically linked the hippocampus to motor sequence learning[58] and to the sleep-dependent consolidation of these tasks.[8,9] Furthermore, since the complex sequence of rungs was learned in a novel environment (mice were not exposed to the wheel before), a subset of more "plastic" hippocampal place cells may have been engaged due to the increase in environment/cue complexity.[59] In summary, the strong involvement of both motor cortex and hippocampus in mice seems to support these conclusions.

A previous study in humans found that the overnight gain in performance after training in a motor sequence task was limited to fast learners and not found in slow learners.[36] The same study found that fast and slow learners recruited different neural systems during training—hippocampus and cerebellum, respectively—suggesting that sleep effects may also depend on the specific neural networks engaged during training. We found differential effects of sleep based on performance, although both fast and slow learners improved after sleep. In fast learners, sleep consolidated motor memory by stabilization that is by preserving the skills learned during the first session. This result is in line with the evidence for sleep-dependent consolidation in rodents in various hippocampus-dependent tasks, including contextual fear conditioning,[60–62] radial arm water maze,[63,64] Morris water maze,[65] reversal learning of Y maze,[66] and novel object-place recognition.[67] Using these tasks, sleep-dependent stabilization was documented both in mice[60,66] and rats,[62–65,68,69] since at the beginning of the retest session memory was impaired after SD but preserved after sleep. We also found, however, that longer sleep correlated with one measure of offline gain, as well as with the mean performance in the second session. Thus, at retest, performance in our sleep and SD mice may have differed not only because of the deteriorating effects of SD but also due to a direct positive effect of sleep. Among the slow learners, performance did not get worse after sleep loss, perhaps because it was already low at the end of the first session. Sleep, however, led to an offline gain, although we could not find any correlation between this effect and time spent asleep after initial training. One study in humans found a correlation between offline gain in performance of motor sequence learning and the amount of stage 2 NREM sleep specifically during the last quarter of the sleep period.[2] Thus, we may have missed the correlation because we could only assess total sleep duration.

Our mice showed prominent interindividual variability in absolute levels of performance and performance improvement across sessions. The correlation between sleep and subsequent performance in fast learners may account for some of the interindividual variability among the S group. Still, several sleeping mice showed little or no improvement, or even worse performance after sleep, suggesting that sleep is only one of the factors affecting memory consolidation in this task. Of note, in contrast to previous studies that allowed free access to the complex wheel before training,[26–30] our mice were subjected to intense training in a short period of time and without prior habituation to the wheel. Thus, interindividual differences in the stress response to a novel and challenging environment may have contributed to some of the variability. Also unclear are the reasons for the interindividual variability after SD: more SD mice than sleep mice showed lack of memory consolidation across sessions, but many SD animals performed at retest as well as sleep mice. In humans, there are stable, trait-like differences in the susceptibility to cognitive impairment caused by acute SD or chronic sleep restriction,[70–72] which are at least partially attributable to genetic background.[73] Our mice, however, shared the same genetic background and thus other factors must be involved. In humans, neuroimaging studies found that differences in the activation of frontoparietal regions during a working memory task at rest are associated with differences in the extent of the cognitive decline during SD.[74,75] Moreover, recent evidence suggests that differences in the microstructure of the white and grey matter can underlie the interindividual differences in the resistance to sleep loss.[76–78] To our knowledge, there are no studies in sleep-deprived rodents focusing on interindividual differences and their underlying mechanisms.

In summary, our results show for the first time in mice that sequence learning benefits from sleep, while rotarod training, an easier task that is associated with less pronounced activation of motor cortex and hippocampus, does not. We also show for

the first time in mice, where genetic factors are easier to control, that the effects of sleep and sleep loss greatly vary from mouse to mouse. This interindividual variability, which is increasingly being recognized in humans, strongly suggests that factors other than sleep must modulate memory consolidation in the first crucial hours that follow learning.

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SUPPLEMENTARY MATERIAL

Supplementary material is available at *SLEEP* online.

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